

THE MECHANISM OF THE SHADOW REFLEX IN CIRRIPIEDIA.

I. ELECTRICAL ACTIVITY IN THE SUPRAESOPHAGEAL GANGLION AND OCELLAR NERVE¹

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The protective withdrawal-closure reaction of barnacles in response to shading was first alluded to by Coldstream (1836), who noted that *Balanus* would close when the hand was passed over it at a distance of twelve or fourteen inches and wrote ". . . we could not but conclude that the animal was made sensible through the medium of the air, of the presence of some foreign body, and fearing danger, closed its shell for self-protection . . ." (p. 688). Von Siebold (1848, p. 343) correctly surmised that this reaction was caused by shading, but at the time denied the existence of eyes in adult cirripedes. Leidy (1848) discovered ocelli in *Balanus rugosus* (probably *B. crenatus*), and Darwin (1851, 1854) described these structures in other balanoids and in *Lepas anatifera*. Gruvel (1893) produced a rather thorough study of the morphology of the nervous system of *L. anatifera*, including considerable detail on the eye and associated ganglia, and Nussbaum (1890) noted the occurrence of a pair of ocelli in *Mitella* (= *Pollicipes*) *polymerus*.

Since Leidy's discovery of the ocelli in adult barnacles, it has been assumed that they were the sole photoreceptors and thus instrumental in the shadow reflex. The only attempt to establish the involvement of the ocelli in the reflex, however, is that of Fales (1928) who reported that the ablation of these structures in *Balanus cburneus* extinguished the reflex. Because of their internal location considerable injury must have occurred during removal, and the failure to respond may have had other causes.

The behavior of *Balanus* in response to shading under various conditions of illumination and other environmental factors was extensively reported by von Buddenbrock (1930), who pointed out that there is only a response to a sudden decrease in illumination, and that there is no overt short-term response to an increase in illumination.

The existence of a stereotyped behavioral response of the pure "off" type, coupled with what appeared to be a few millimeters of primary sensory neuron (Gwilliam, 1962) between photoreceptor and ganglion, suggested cirripedes as being good material in which to attempt an analysis of a behavioral response from primary receptor to the motor neurons activating the muscles involved. The

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recent report of Hoyle and Smythe (1963) on the giant muscle fibers of barnacles also suggests the possibility of intracellular recording of muscle potentials while the animal is being subjected to sudden changes in illumination.

This paper reports the results of a survey of four species of barnacles and represents the beginning of what is hoped will eventually be an analysis of the various factors involved in the shadow reflex from a neurophysiological point of view. A preliminary account of some parts of this work has been reported (Gwilliam, 1962).

MATERIALS AND METHODS

The following species of barnacles were used in the study: *Mitella* (= *Pollicipes*) *polymerus* (Sowerby), *Lepas anatifera* L., *Balanus cariosus* (Pallas), and *Balanus cburneus* Gould. *Mitella* and *B. cariosus* were collected as required from the central Oregon coast. *Lepas anatifera* and *B. cburneus* were provided by the Supply Department of the Marine Biological Laboratory, Woods Hole, Mass.

Details of the connections between photoreceptors and the supraesophageal ganglion were determined by dissection and methylene blue staining after it was ascertained physiologically that the structure in question was indeed the photoreceptor.

Electrical activity was recorded with platinum or platinum-iridium hook electrodes or with forceps electrodes. Signals were amplified with a conventional A. C. preamplifier and displayed on one beam of a Tektronix Type 502 dual beam oscilloscope. The second beam of the oscilloscope displayed the signal from a selenium photocell that was placed close to the preparation. Permanent records were made on a moving film with a Grass C-4 kymograph camera.

Shadows were cast with a sector disc driven by an electric kymograph motor, with a 35-mm. camera focal plane shutter, and manually. The light source used was a 6-volt tungsten lamp which was not controlled for absolute intensity, and the distance from the preparation was held constant only during a given experiment. Neutral density filters were used to vary intensity, as well as being used to cast shadows.

For medium bathing the preparation consisted either of sea water or *Balanus* Ringer (Hoyle and Smyth, 1963). Under these conditions and at room temperatures of 18–23° C. preparations were consistently viable for up to 12 hours without special precautions being employed. However, no single preparation was used for more than about three hours.

RESULTS

Structure

Figure 1 illustrates the relationships of the various photoreceptors to the supraesophageal ganglion in the species examined. The histological details of the photoreceptors themselves differ from species to species and will be reported in a separate communication. Some histological details of the eye of *Lepas anatifera* have been reported by Gruvel (1893), and of *Balanus cburneus* by Fales (1928).

As can be seen in the diagrams, the photoreceptor lies in the midline in all the species examined except *Balanus cburneus*. In this species the pair of ocelli

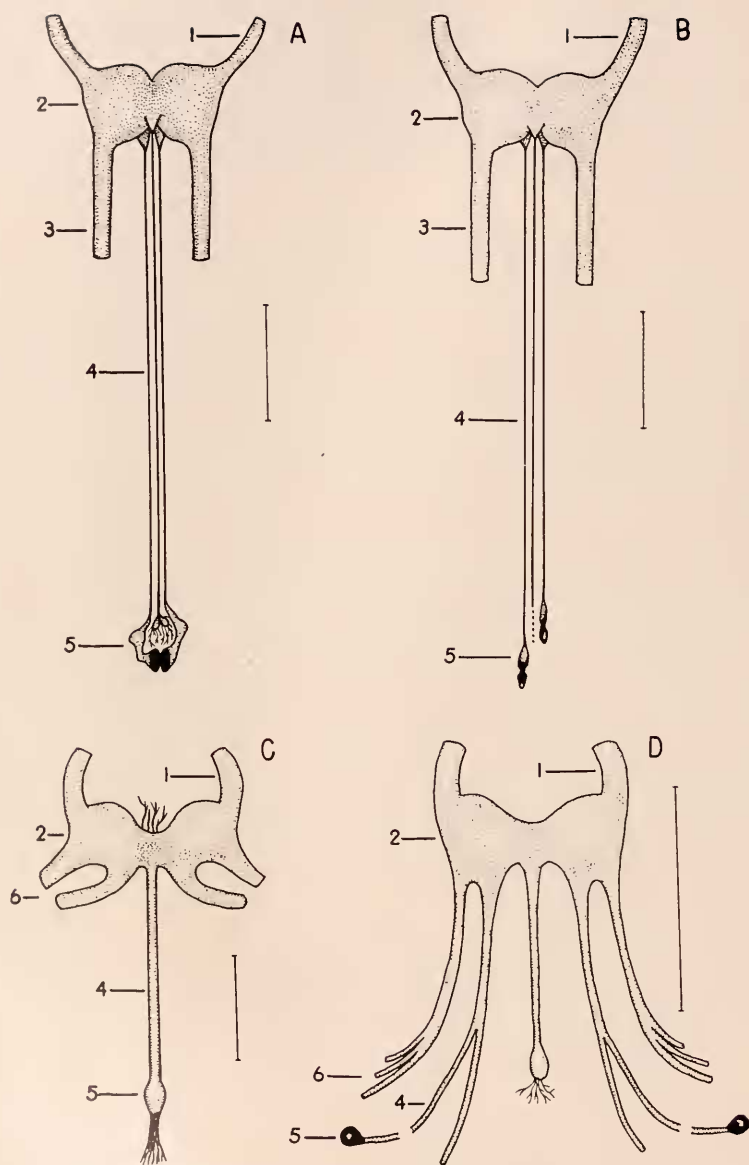


FIGURE 1. Diagrams of the relationship of the ocellus and the supraesophageal ganglion in the four species of barnacles studied. A, *Lepas*; B, *Mitella*; C, *Balanus cariosus*; D, *B. cburneus*. (1, circumesophageal connective; 2, supraesophageal ganglion; 3, stalk nerve; 4, ocellar nerve; 5, photoreceptor; 6, mantle nerves). Gap in ocellar nerve in D indicates the omission of 2-3 mm. of nerve. Scale markers = 1 mm.

are to be found in the mantle lateral to the body, just proximal to the edge of scutes. They are thus located in the inner lining of the shell "looking inward."

Electrical activity

(a) *Mitella polymerus*. Recordings from the stalk nerves of this species in light or after 15 minutes in the dark show a decidedly rhythmical pattern of bursts of activity as illustrated in Figure 2, A. Bursts often occur more frequently than those illustrated, but the general nature of the rhythmical activity is always similar. If a shadow is cast on the preparation in light just after one of these spontane-

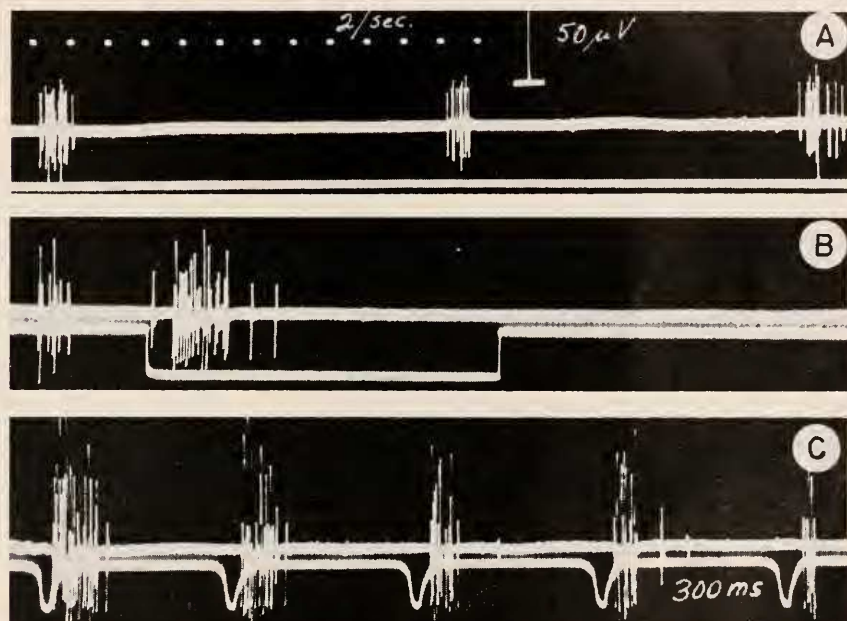


FIGURE 2. *Mitella*. Electrical activity recorded from the stalk nerve. A, Spontaneous activity in the dark. B, The "off" response. C, The effect of multiple shadows of 300-millisecond duration. In this record a response was still evident after 30 such shadows. Downward deflection of lower trace indicates "off," upward deflection "on" in this and all records to follow.

ous bursts, there is a more prolonged response, followed by a longer than expected period of silence, and no activity at the cessation of shading (Fig. 2, B). Multiple shadows of 300 milliseconds duration at two-second intervals elicited a response at each shadow, but adapted somewhat with succeeding stimuli although a definite response was still evident after 30 such shadows (Fig. 2, C). Two hundred millisecond shadows at 2.5-second intervals failed to give repetitive responses, and 100 millisecond shadows at one-second intervals gave a significantly smaller initial response and failed to summate. Shadows of shorter duration failed to elicit any response. The relationship between shadow duration threshold and light intensity was not investigated in this species.

The fact that shadow "intensity" and magnitude of the response are related is illustrated in Figure 3. Shadows were cast with gelatin neutral density filters having per cent transmission characteristics indicated in the upper right hand corner of each record. It can be seen that cutting out 60% of the incident light results in what is essentially a full shadow reflex (compare Figure 3, C and F).

(b) *Lepas anatifera*. This species exhibited responses similar to those of *Mitella polymerus*, but it was also possible to record directly off the ocellar nerve in *Lepas* (Fig. 4). Records from the circumesophageal connectives and stalk nerves showed considerable spontaneous activity, but there was none of the regular rhythmic activity of the type shown by *Mitella* stalk nerves. This may reflect the rather different behavior patterns of the two animals. *Mitella* is a barnacle that lives attached to rocks, along with *Mytilus californianus*. It feeds by extending the cirri into a moving stream of water during high tide and is exposed at low water, during which time the cirri are inactive. Even during feeding, *Mitella*

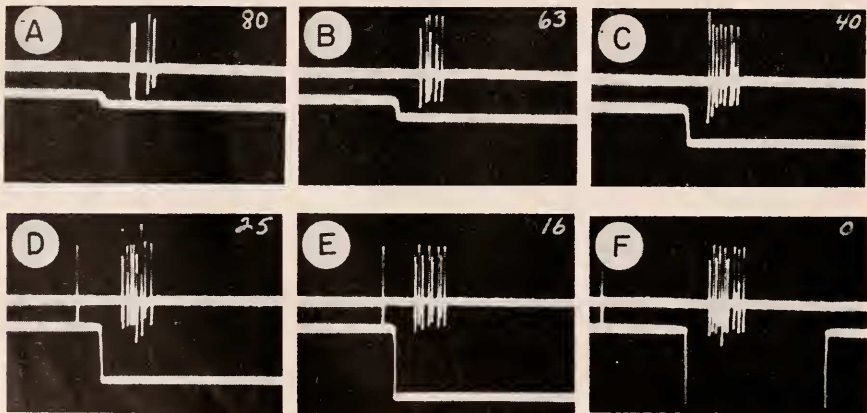


FIGURE 3. *Mitella*. The effect of casting shadows with neutral density filters. Figures in upper right hand corner of each record indicate per cent transmission of the filter used to cast the shadow. Recorded from the stalk nerve.

does not exhibit the regular extension and retraction "fishing" activities so typical of sessile barnacles, and it is possible that the regular rhythmical activity observed in the stalk and mantle motor nerves serves to provide regular pulsations of the muscles that aid in blood circulation in these heartless crustaceans. *Lepas*, on the other hand, lives attached to ships and floating timber and is at all times submerged. *Lepas* feeds much as does a sessile barnacle, by the regular extension and retraction of the cirri, and this activity could serve as a blood pump.

A shadow cast on a preparation such as that illustrated by Figure 4 elicits a burst of large-fiber activity in the circumesophageal connective (Fig. 4, A) and in the stalk nerves (Fig. 4, B, lower trace). A small positive deflection is recorded in the ocellar nerves proper at "off," and at "on" a much larger slow response, referred to as the electroretinogram (ERG), can be recorded (Fig. 4, C) which is graded and decremental. On no occasion have I been able to record spikes from the ocellar nerve.

Early in the investigation spikes were frequently recorded in the *bundle* bearing the ocellar nerves, but careful observation and dissection revealed three trunks in the bundle, only two of which led from the ocelli (Fig. 4, diagram). By recording from these trunks one at a time, it was established that the middle trunk gave rise to the spikes while the ocellar nerves gave only the ERG. If this middle nerve was severed at the level of the supraesophageal ganglion, the activity was arrhythmic and unaffected by light or shadows. If recordings were made on the intact nerve, a "shadow reflex" was evident.

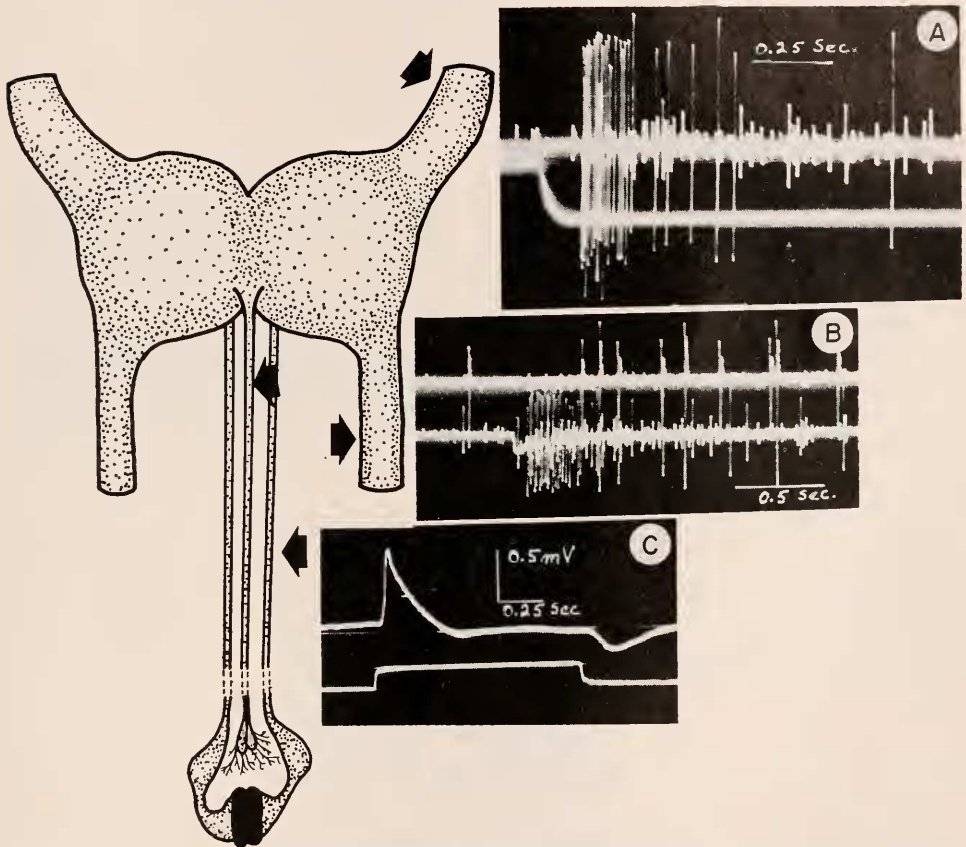


FIGURE 4. *Lepas*. Electrical events recorded from various positions in the nervous system. Arrows indicate recording sites. See text for explanation.

While the above seemed to rule out any possibility of this median nerve being responsible for the activity observed in stalk nerves and circumesophageal connectives, a further experiment was performed to establish the point. Leaving the median nerve intact, one could record from it and the stalk nerves simultaneously. A shadow was cast, and the resulting record demonstrates that the activity in the stalk nerve following shading (Fig. 4, B, lower trace; downward deflection of baseline signals "off") could not have been initiated by the activity in the

median nerve (Fig. 4, B, upper trace) because it precedes it in time. Further, severing only the ocular nerves while leaving the median nerve intact abolishes the activity illustrated in Figure 4.

(c) *Balanus cariosus*. The photoreceptor in this large sessile barnacle consists of a ganglion lying close to the adductor scutorum muscle and connected to the supraesophageal ganglion (Figs. 1 and 5). It lacks the obvious ocellar pigments seen in other barnacles and differs very little in evident color from the rest of the nervous system in that region. Its function as the photoreceptor was established experimentally, and no other structure serving this function has been located in this species.

Considerable time was spent searching for an obvious ocellus, but none was found. The animal has a perfectly good shadow reflex, however, so that it was evident that a photoreceptor was present. To locate the structure, a circum-

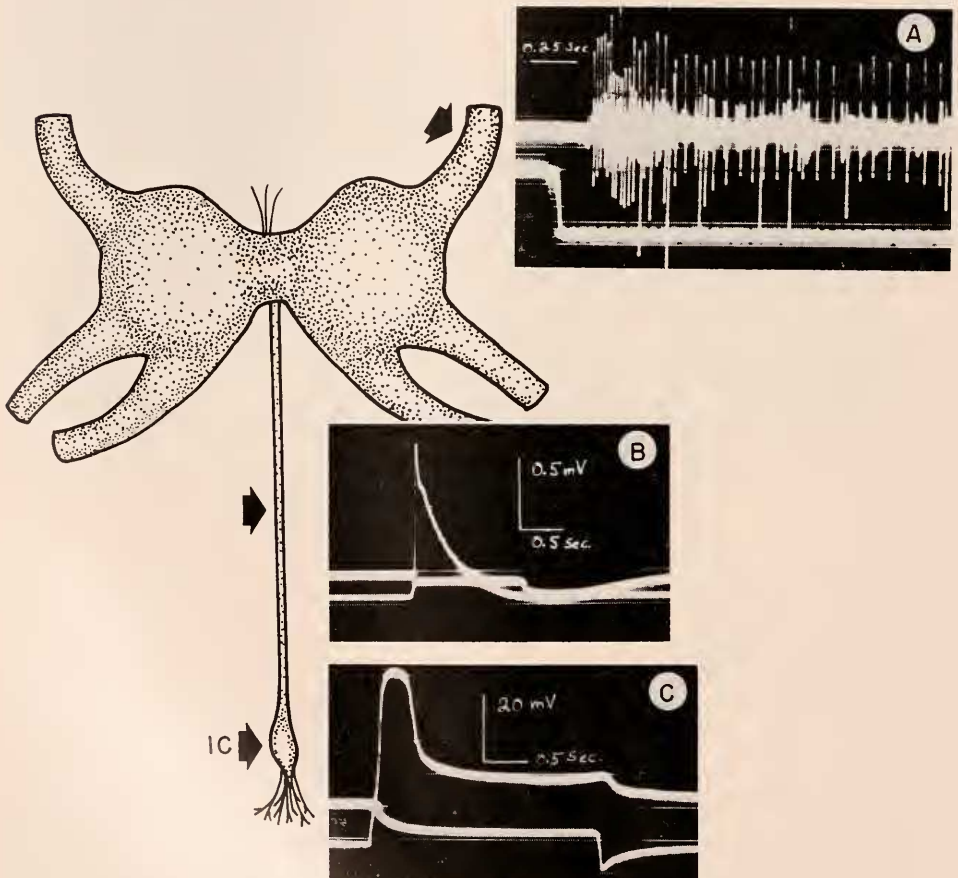


FIGURE 5. *Balanus cariosus*. Electrical activity recorded from the sites indicated by arrows. IC indicates record C is a presumed intracellular response. A and B, A. C. recording; C, D. C. recording.

esophageal connective was placed on a recording electrode, and the preparation was tested for the "off" response, then small areas were shaded, the general area of sensitivity quickly located, and the small ganglion was seen. Then, using a needle point to create a small shadow, it was demonstrated that shading the ganglion and only the ganglion gave the response. Crushing the ganglion or severing the tract to the supraesophageal ganglion obliterated the response in the circumesophageal connectives.

Under the recording conditions there was considerable small-spike spontaneous activity recorded from the circumesophageal connectives in *B. cariosus*. When a shadow was cast on the preparation there was a burst of large-fiber activity that persisted for a considerable period (Fig. 5, A) and ceased abruptly at "on." If left in the dark, the "shadow reflex" burst persisted, gradually diminishing in frequency for four to ten seconds.

Recordings from the ocellar nerve freed of other fibers always resulted only in an ERG similar to that recorded from the ocellar nerve of *Lepas* (Fig. 5, B).

At this time several attempts were made to penetrate cells in the photoreceptor ganglion with glass microelectrodes, and on a single occasion this appeared to be successful. One of the records obtained is reproduced as Figure 5, C. An attempt was made to record under varying light intensities, but I was unable to hold the cell for a sufficient length of time. The absence of spikes in this record supports the idea that these cells do not generate propagated action potentials, but under the circumstances this cannot be taken very seriously. Failure to hold the cell may indicate injury, and a failure to repeat the penetration has made it impossible to gain further information. The absence of spikes in such a record may indicate injury or that spike generation takes place in the axon and does not invade the soma. The electrode was tested with light flashes before and after the record was made to be certain the potential was not due to the action of light on the electrode itself.

Figure 6 illustrates the relation between ERG amplitude (recorded from the severed ocellar nerve) and light intensity. It further emphasizes the fact that the primary event is an "on" response, while the behavioral event takes place only at "off." This suggests that the activity of the photoreceptor transmitted via the ocellar nerve inhibits cells in the supraesophageal ganglion, and this inhibition is released at "off."

Failure to record spikes from the ocellar nerve was originally viewed simply as a technical failure in recording. However, after numerous attempts at recording under various conditions (in air, in oil, with partially dried preparations to minimize shunting, in carefully subdivided bundles), I was forced to consider the possibility that propagated spikes did not occur in the ocellar nerve and that the information was transmitted via electronic spread. Histological examination of the photoreceptor ganglion and the axons leading to the supraesophageal ganglion indicates that there are about eight cells present in the ganglion, and no synapses occur until the supraesophageal ganglion, which may be as distant as 4 mm. in a large individual. This is a very long distance for the phenomenon of electrotonic spread to be effective, but the following experiment suggests that this is indeed the case.

In *Balanus cariosus* it is possible to make a preparation in which the supra-

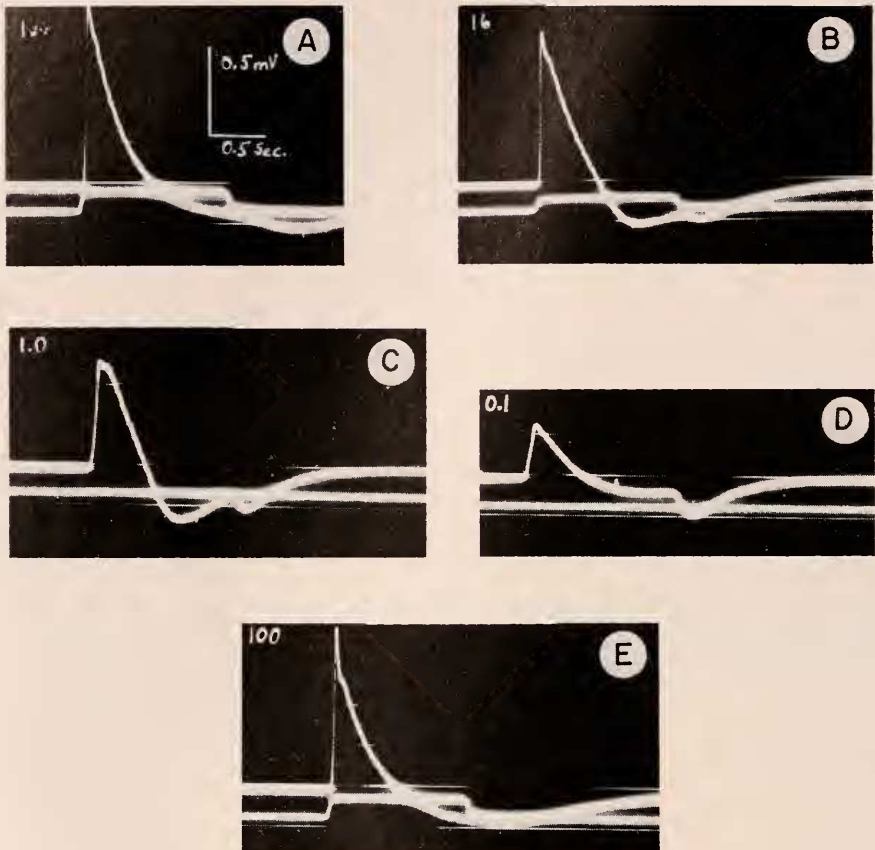


FIGURE 6. *Balanus cariosus*. The effect of light intensity on the externally recorded ERG. Recorded from the severed ocellar nerve. Per cent transmission of light flash indicated in upper left hand corner of each record. Photocell monitor failed to record in C and D. A. C. recording.

esophageal ganglion is cut free from the body of the barnacle, leaving it attached only by the ocellar nerve. The supraesophageal ganglion can then be lifted free of the bathing medium while the photoreceptor ganglion is still immersed, and recording can be accomplished from the circumesophageal connective. The supraesophageal ganglion is kept viable either by lifting into oil or preventing it from drying by the application of bathing medium at intervals.

In this way it was possible to soak only the photoreceptor ganglion and its axons in a solution of procaine, and test periodically for the shadow reflex in the circumesophageal connectives. Then the supraesophageal ganglion could be lowered into the procaine and testing continued. The results of such an experiment are shown in Figure 7. Soaking the photoreceptor ganglion in 0.5% procaine for as much as 20 minutes (Fig. 7, A, B) did not abolish the shadow reflex, but within two minutes after lowering the supraesophageal ganglion into the procaine,

spiking was virtually abolished (Fig. 7, C). The effect was reversible, for 30 minutes after washing, a shadow reflex was again obtainable (Figure 7, D).

This experiment establishes that procaine does prevent spiking in barnacle nerves, and other studies (*e.g.*, Katz, 1950) indicate it does not prevent generator potentials. The experiment does not prove that electrotonic spread is the mechanism for transmission, for there is no direct evidence that the procaine penetrates the photoreceptor ganglion. There is little reason, on the other hand, to assume procaine does not penetrate, for the receptor ganglion sheath is, if anything, less well developed than that around the supraesophageal ganglion. Cut ends of nerves leading to the supraesophageal ganglion may speed penetration in that region, so the experiment can only be regarded as supporting, but not proving, the hypothesis of electrotonic spread.

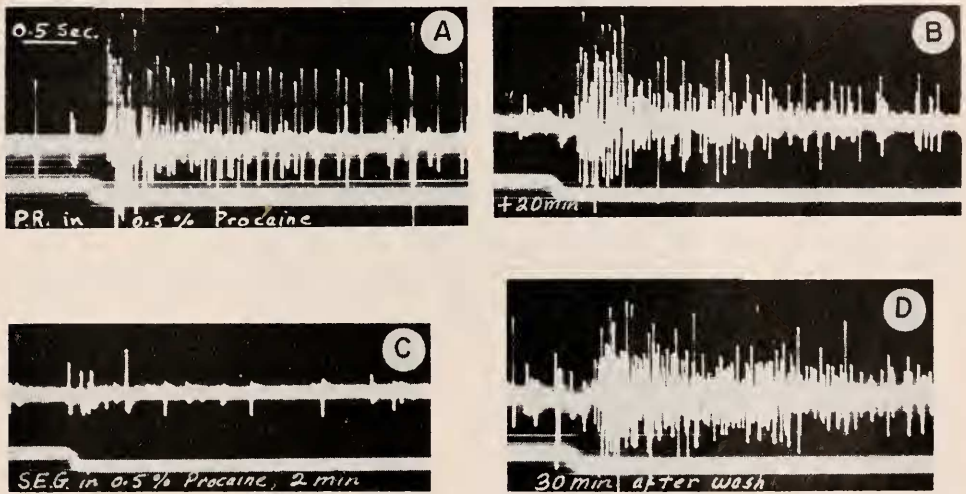


FIGURE 7. *Balanus cariosus*. Results of the procaine experiment described in the text. PR, photoreceptor; S.E.G., supraesophageal ganglion.

(d) *Balanus cburneus*. These are the smallest of the barnacles on which recording was attempted, but the laterally located photoreceptors were sufficiently interesting to make the attempt worthwhile. The general structure of the ocellus of *B. cburneus* is reported by Fales (1928), who illustrates the two sensory cells in each. These cells attain a size of 100 microns and appear to send their axons directly to the supraesophageal ganglion without synapses. There is also a median ganglion present as in *B. cariosus* which has photoreceptor activity.

Figure 8, A, B illustrates the activity recorded from the circumesophageal connective when a shadow is cast, and its cessation when the preparation is exposed to light. If the preparation is kept in the dark, the activity continues for about 40 seconds, decreasing exponentially in frequency from ca. 80/sec. in the first second to ca. 3/sec. in the last second. If multiple shadows are cast, bursts will continue to occur for more than 30 shadows. If, however, activity is recorded in the adductor motor nerve which originates in the ventral ganglion, there is a

failure after about four similar shadows. This is in contrast to *Lepas*, where adaptation to multiple shadows appears to take place at the level of the supraesophageal ganglion rather than the ventral ganglion (Gwilliam, 1962).

Records taken from close to the ocellus result in an ERG similar in form to the others already noted, and a similar response is recorded from the median nerve (Fig. 8, C, D).

The fact that the median nerve in this species serves as a photoreceptor was discovered by accident. During a routine experiment to establish the obvious lateral ocellus as the sole photoreceptors, recordings were taken from the circumesophageal nerve before and after severing the ocellar nerve at the level of the

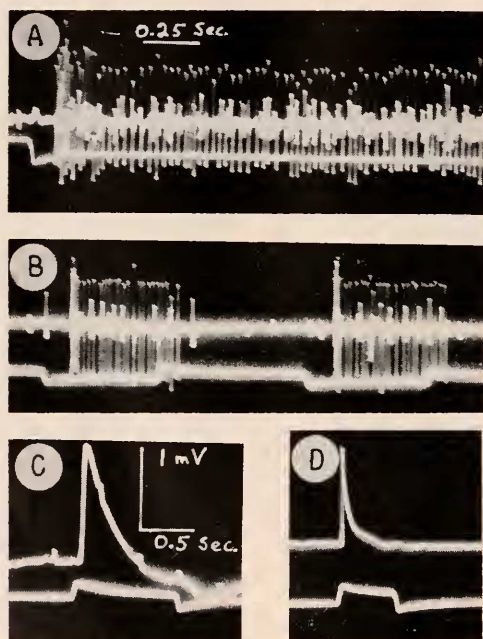


FIGURE 8. *Balanus cburneus*. Electrical events recorded from the circumesophageal connective (A and B), the ocellar nerve (C), and the "median" nerve (D). Time marker in A applies also to B; time and amplitude in C applies also to D. A. C. recording.

ocelli, and a good shadow response was evident (Fig. 9, A). Then, all four lateral nerves were severed at the ganglion (the circumesophageal connectives having been severed previously), and the median nerve was cut *distal* to the small ganglion. Under these circumstances, a good shadow reflex was still obtainable (Fig. 9, B, C). The median nerve was then severed just proximal to the small distal ganglion, leaving a length of nerve which apparently itself was photosensitive, for a good response was still observed (Fig. 9, D). Finally, cutting the median nerve at the level of the supraesophageal ganglion abolished the response (Fig. 9, E).

The function of this small median ganglion as a photoreceptor is not universal in *B. cburneus*, for not all preparations show this activity. It is even less common

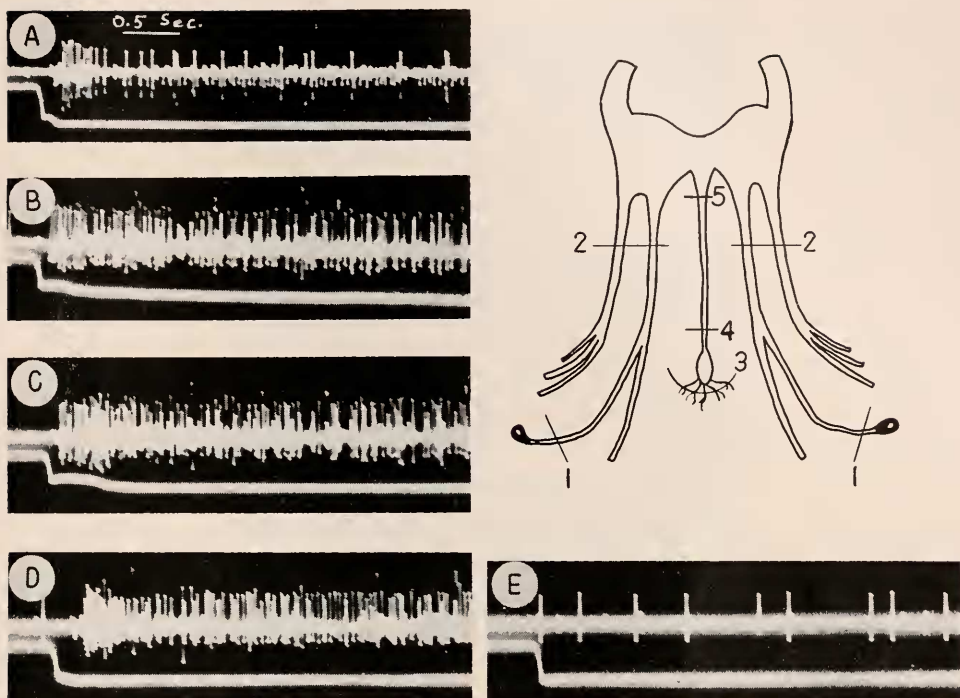


FIGURE 9. *Balanus eburneus*. The median distal ganglion and nerve functioning as a photoreceptor. Numbers on the diagram indicate the order of severing nerve tracts. A was recorded following cut 1, B following cut 2, etc. Circumesophageal recording.

for the median nerve itself to function in this way, but it is a factor which must be considered in ocellar ablation experiments.

If recordings are made from the entire severed bundle containing the ocellar nerve at the level of the supraesophageal ganglion (*i.e.*, preganglionic but with all distal structures including the ocellus intact), large spikes are often observed that frequently commence firing at "on" (Fig. 10, A). Such spikes have never been recorded close to the ocellus where only the ocellar nerves are present, but it is conceivable that the spiking locus is at some distance from the sensory cells.

While these potentials have not been completely ruled out as being activity from the photoreceptors, the following observations suggest that this is due to the activity of some other sensory system, possibly a mechanoreceptor.

- (a) The activity occurs spontaneously at times in the absence of light (Fig. 10, B).
- (b) The activity does not occur at "on" if all other input to the supraesophageal ganglion is interrupted.
- (c) The activity frequently does not cease at "off" as would be necessary for the second order "off" response to occur (Fig. 10, C).
- (d) Cutting the ocellar nerve at the ocellus does not abolish the spikes.
- (e) Immersing the preganglionic fibers in procaine does not abolish the "off" response in the circumesophageal connectives.

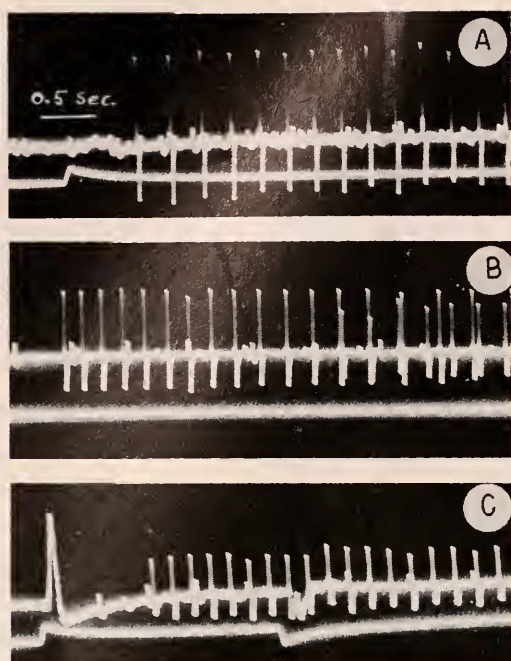


FIGURE 10. *Balanus crenatus*. Large-fiber activity from nerve tract containing the ocellar nerve recorded preganglionically. A, The "on" response. Note long latency. B, Spontaneous activity in the dark. C, Another "on" response with long latency and exhibiting the failure to stop at "off." Note elements of the ERG at "on" and "off."

It seems probable, therefore, that this fiber is involved in some other yet unknown sensory system.

DISCUSSION

Despite differences in detail of photoreceptor position and structure, all of the barnacles described in this study have essentially similar reactions at comparable points in the nervous system. This result is to be expected on the basis of the simple behavioral response common to all. As a result of this similarity, it is possible to generalize the discussion, pointing out differences where they occur and seem significant.

Beginning with the sensory output, all the evidence points to an "on" response, as would be expected from a photoreceptor, but this response takes the form of a simple electroretinogram consisting of a relatively large negative-going slow potential at "on" (from an active lead on the ocellar nerve with reference to an indifferent electrode in the medium) and a small positive deflection at "off." These ERGs were A. C.-recorded, and the form under these conditions may be interpreted as an initial large depolarization which quickly drops to a lower level that is maintained in the light. Upon cessation of illumination, the potential returns to the resting level, giving a small positive wave when A. C. recording is used.

An electroretinogram of this form is essentially the same as those recorded

from insect retinular cells (Naka, 1961), *Limulus* retinular cells (Fuortes, 1958), and corresponds to the interpretation of the insect dorsal ocellus ERG given by Ruck (1961) without the complicating factors of the synaptic layer and post-synaptic ocellar nerve, *i.e.*, a transient "on" response and a sustained depolarization in light. The single presumed intracellular record from *B. cariosus* supports this interpretation, but under the circumstances it cannot be regarded as decisive.

The evidence also indicates that this ERG is the only form of photoreceptor activity transmitted to second order neurons in the supraesophageal ganglion and acts in an inhibitory fashion in these cells. Such a system would correspond to that described by Ruck (1962) as (p. 632) ". . . The general, perhaps evolutionarily primitive, synaptic relationship between retinula cells and the second order neurons. . . ."

The recent report of Naka and Eguchi (1962), however, will not permit the generalization that retinula cells do not generate spike potentials, for these authors have demonstrated that spikes do occur in retinula cells in the lateral eye of the drone honeybee. As yet there are no published intracellular records from the insect ocellus or indeed from the ocellus of any arthropod, and it is possible that in the more simply organized of these structures, the generator potential is the only form of activity. The evidence presented here suggests that this is the case in the very simple photoreceptor of barnacles. The single intracellular recording obtained matches closely in form those obtained from the worker honeybee (Naka and Eguchi, 1962) and the same explanations these authors offer for failure to record spikes may hold. However, the failure to record spikes in the ocellar nerve and the failure of procaine to obliterate the circumesophageal "off" response lends support to the idea that the barnacle photoreceptor cells do not generate all-or-nothing action potentials.

It should be noted that the barnacle ERG indicates a depolarization at "on," not the reverse as shown by Parry (1947) for the ocellar nerve of *Locusta*, even though both organisms show an "off" response in the circumesophageal com-mixtures. Hoyle (1955) later demonstrated action potentials in the ocellar nerve of *Locusta*, and Waterman (1953) in the median ocellar nerve in *Limulus*. However, the insect ocellar nerve is composed of second order neurons, and the "off" response may be explained just as is the "off" response in the circumesophageal connectives in the barnacle. In the *Limulus* ocellus the origin of the spike potentials is obscure (Waterman, 1953).

The barnacle photoreceptor is presumably derived from the median naupliar eye, not the compound eyes of the cypris (Fales, 1928; Doochin, 1951). As such, its original position in the larva, lying physically very close to the supraesophageal ganglion, would render a mechanism of electrotonic spread quite plausible, just as in the insect dorsal ocellus where the first synapse is very close. The development of the adult eye might then be envisaged as involving an elongation of the photoreceptor cell axon as the ocellus assumed the new position, but retaining the "primitive" mechanism.

If the functional interpretation presented here is correct, and if there are indeed no synapses between the photoreceptors and the supraesophageal ganglion, then the barnacle "eye" is perhaps the simplest readily accessible photoreceptor yet known. The primary cells are few (two in each ocellus of *B. cburneus*, six

to nine in the photoreceptor ganglion of *B. cariosus*, two in each ocellar ganglion of *Lepas*) and of relatively large size, and thus they offer an opportunity to study the primary receptors in isolation. We are currently engaged in attempts to record intracellularly from the large cells in the ocellus of *Balanus eburneus*.

Next, examining the output of the supraesophageal ganglion as recorded in the circumesophageal connectives in the two species of *Balanus*, we find that relatively few large cells respond at "off" in a tonic fashion, and this response does not adapt with multiple shadows. In *Lepas*, on the other hand, adaptation occurs relatively rapidly at this level (Gwilliam, 1962), as can be ascertained from recordings of the output at both the circumesophageal connectives and the stalk nerves. This may be a consequence of the fact that the stalk nerves originate in the supraesophageal ganglion and probably represents at best the third neuron in the chain, while in *Balanus* the nerve serving the adductor muscle originates in the ventral ganglion. When the motor output from the ventral ganglion to the adductor scutorum muscle in *B. eburneus* is examined, a rapid failure to respond to multiple shadows is seen. The difference in the recordings from the circumesophageal connectives in the two types of barnacles is not so readily explained but may have to do with the detailed distribution of motor cells. However, the fact that adaptation occurs just prior to the motor output stage would permit the barnacle to ignore repeated inconsequential shadows that must occur constantly while the animal is feeding in nature, but still be receiving information in the central nervous system about the changing light level in the environment.

In *Mitella* the failure of the motor output to the stalk nerves to adapt to multiple shadows is puzzling in the light of the rapid adaptation of the motor output of the other barnacles. The situation illustrated in Figure 2, C, however, is not always the case, for at different shadow durations and frequencies of shadow-casting adaptation does occur. It is possible that in this particular instance the spontaneous rhythm has been reset.

SUMMARY

1. The electrical activity of various parts of the nervous system under changing conditions of illumination in four species of barnacles has been studied.

2. The primary event at the photoreceptor is an "on" response that takes the form of a simple ERG similar to that described for the retinula cells in a number of other arthropods.

3. Evidence is presented that suggests that this information is transmitted to the second order neurons in the supraesophageal ganglion via electrotonic spread, but this cannot as yet be regarded as conclusive.

4. The second order neurons respond in a pure "off" fashion and are inhibited at "on." In *Balanus* this response, monitored at the circumesophageal connectives, is tonic, while in *Lepas* and *Mitella*, recordings at the same position display a phasic response. It is suggested that, at best, third order neurons are responsible for the phasic response in the latter two genera.

5. Evidence is presented that both the primary "on" response and the second order "off" response are graded with intensity of illumination.

6. Failure to respond to multiple shadows is evident in the motor output of all species except *Mitella*, in which the stalk nerves respond repeatedly to multiple

shadows. A possible explanation for the latter and a possible advantage of the former is offered.

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